THE OSMOTIC RELATIONS BETWEEN WHITE AND YOLK IN THE HEN'S EGG

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(From the Marine Biological Laboratory, Plymouth.)

(With One Text-figure.)

THE osmotic relations between white and yolk in the fertilised and unfertilised hen's egg have often been investigated. The complexity of these relations is presumably due largely to peculiar physico-chemical properties of the colloid constituents of the egg. The purpose of this paper is primarily to confirm the existence of a considerable osmotic difference between white and yolk in the unfertilised hen's egg, in reply to a recent denial, and then to describe some preliminary experiments on the state of water and of several dissolved substances in the egg. The bearing of these experiments on the relations between white and yolk in the intact egg will then be discussed briefly.

The fact that egg-yolk appeared to freeze at about − 0·56° C. while the white froze at − 0·46° C. was demonstrated in 1912 by Bialascewicz, though apparently familiar to several earlier workers. It only became a matter of general interest when Straub (1929) suggested, on experimental grounds, that this freezing-point difference was maintained by the performance of osmotic work by the histologically structureless vitelline membrane, supposed formerly to be merely an inert membrane separating two phases. As Straub's work is discussed in greater detail by Needham and Smith (see Needham *et al.*, 1931), it need only be added here that following Hill's demonstration (1930 *a*) that the work, if it is performed at all, depends on anaerobic processes, Needham and his collaborators (1931) published a detailed investigation from which Straub's idea of a steady state involving the performance of work became very improbable, although an explanation of the phenomenon (or rather of the slowness with which it disappears) in terms of known physical properties of yolk, white and vitelline membrane is not yet apparent. One explanation, due to Grollman (1931), is that the apparent osmotic difference between white and yolk is merely a result of experimental errors in the determination of freezing-point or vapour pressure of yolk. The incorrectness of this explanation would follow almost certainly from Needham's work and no further vindication of the freezing-point method was necessary. It seemed advisable, however, to reply to Grollman's vague criticism of Hill's vapour-pressure method (1930 *b*) when used with viscous materials such as egg yolk.

Grollman remarks that differences in thickness of the liquid films on the thermopile faces might be the cause of "irgendeine Asymmetrie", which can only be taken to mean that the system was not in equilibrium when readings were taken, since in an equilibrium measurement the final results should not be dependent on the thickness, viscosity, specific heat, etc., of the substances used. These factors can only affect the *rate* of equilibration. The experiments in the next section were designed to show that a steady state is already reached when measurements are made.

EXPERIMENTS ON THE VAPOUR-PRESSURE DIFFERENCE BETWEEN WHITE AND YOLK

Materials and methods require little description. The vapour-pressure determinations were made with four (sometimes only two) small thermopiles of recent design (see Hill, 1931, p. 44, footnote) which gave steady galvanometer readings less than 20 min. after setting up. The galvanometer used was a Zernicke "Zc" moving coil instrument. The details of temperature regulation, etc., for the thermostat were as described in earlier papers (Hill, 1930 *b* and 1931, p. 40). The eggs used were bought as fresh from a Plymouth shop, so that the age, and therefore the vapour-pressure difference between yolk and white, were variable. The squares of filter paper used were soaked in the viscous yolk for about an hour before use.

The following groups of experiments were made:

(i) The vapour-pressure difference between white and yolk was measured with different quantities of fluid on the thermopile face, the amounts used being varied by placing three squares of filter paper soaked in the fluid instead of the usual one (Table I).

Table I. *Apparent vapour-pressure difference between white and yolk with varying amounts of substance on thermopile surface.*

Columns 1 and 2 show amounts of white and yolk respectively; column 3 is the apparent vapour-pressure difference, in terms of per cent. NaCl.[1]

		1 White	2 Yolk	3 Vapour-pressure difference
1		1	1	0·177
		1	3	0·185
2*		1	1	0·002
		1	3	0·006
		3	1	0 003
3		1	1	0·208
		1	3	0·213
4		1	1	0·197
		1	3	0·204
5		1	1	0·152
		1	3	0·149
		3	1	0·147

* Preserved egg.

[1] Throughout this paper vapour-pressure differences are given as the strength of a sodium chloride solution, expressed in gm. NaCl dissolved in 100 gm. water, which would produce the same

When thick layers were used, the constant reading was attained, with the smallest thermopiles, in about the same time as with the usual thickness, but the readings were very markedly more unsteady. Presumably this effect is due to a lag in the response of one face to small external fluctuations, thus introducing an asymmetry with respect to any disturbance that sends the system momentarily out of equilibrium.

(ii) The results of direct measurement, with white and yolk on opposite faces of the instrument, were compared with those obtained using an intermediate standard salt solution; again the agreement was quite satisfactory, as shown by the data in Table II.

Table II. *Direct and indirect measurement of vapour-pressure differences.*

Column 1: substances on thermopile faces, the measured vapour-pressure difference between these substances being given in column 3. Column 2 gives the strength of the NaCl solutions on the wall of the thermopile chamber. Column 4: the difference between white and yolk computed from the indirect measurements.

	1 Substances on thermopile faces	2 Wall solution (% NaCl)	3 Vapour-pressure difference	4
1	White Yolk	0·7	+ 0·179	—
	White 0·82 % NaCl	0·82	+ 0·097 ⎱	
	Yolk 0·82 % NaCl	0·82	− 0·088 ⎰	0·185
2	White Yolk	1·0	+ 0·332	—
	White 1·0 % NaCl	1·0	+ 0·286 ⎱	
	Yolk 1·0 % NaCl	1·0	− 0 008 ⎰	0·294
3	White Yolk	1·0	+ 0·249	—
	White 1·0 % NaCl	1·0	+ 0·293 ⎱	
	Yolk 1·0 % NaCl	1·0	+ 0·060 ⎰	0·233

In experiment 1 the readings were exceptionally steady and they remained practically constant for 1·5 hours, the values for different thermopiles varying slightly in opposite directions—suggesting that equilibrium had been reached.

(iii) Grollman tried to avoid errors in freezing-point determination of the yolk by dialysing it, in a coarse collodion membrane, against a measured volume of water or a salt solution; he then determined the freezing-point of the dialysate after equilibration and so was able to calculate the original freezing-point of the yolk. The values obtained in this way were exactly the same as the directly determined freezing-point of the white. Some of these dialysis experiments have been repeated, using Whatman parchment diffusion shells instead of Grollman's collodion membranes, and measuring in most cases the original apparent vapour-pressure dif-

galvanometer deflection when opposed on the vapour-pressure thermopiles by distilled water. The units used are thus immediately related to what is actually measured, since the thermopiles are always calibrated with a known sodium chloride solution. This seems preferable to the common habit of converting the quantity measured into "absolute" or "ideal" units such as osmolality, ideal molality, etc. In later tables "freezing-point depressions" are calculated from the vapour-pressure data simply for convenience in comparing the data with freezing-point determinations of other workers, but it should be remembered that the vapour pressure of a substance at 18° C. does not necessarily have any "ideal" or thermodynamic connection with the temperature at which the substance freezes. This is especially true of the complex materials dealt with here (cp. p. 327 and Fig. 1).

ference between white and yolk, the final difference between the dialysates, and that between the dialysed white and yolk. Again all measurements are consistent with the assumption of a real difference of vapour pressure between white and yolk (Table III).

Table III. *Dialysis experiments with egg white and yolk.*

Column 1: time of dialysis in hours.
Column 2: strength of NaCl solution used for dialysis, in per cent.
Column 3: weight of solution used for 10 gm. egg white or yolk.
Column 4: vapour-pressure difference between dialysates, expressed as per cent. NaCl.
Column 5: vapour-pressure difference between white and yolk after dialysis.
Column 6: original apparent difference between white and yolk.
Column 7: original difference calculated from columns 3 and 4 and the determined total water of white and yolk.

	1 Time (hours)	2 Dialysis solution	3 Quantity	4 Difference between dialysates	5 Difference between white and yolk after dialysis	6 Original difference observed	7 Original difference calculated
1	20·0	0·7	2·0	0·096	0·089	—	0·125
2	24·0	11·0	3·0	0·149	0·133	0·201	0·222
3*	14·5	0·7	4·0	0·093	0·113	0·190	0·145

* Duck's egg.

(iv) Grollman dialysed a viscous lecithin emulsion and found that after 50 hours the apparent freezing-points of dialysate and emulsion differed by 0·090° C. This difference was probably due largely to incomplete equilibration. Experiment showed that commercial (B.D.H.) egg lecithin contains a high proportion of osmotically active substances; in a thick emulsion, even after dialysis for 4 days against distilled water, there was a viscous core surrounded by a more fluid layer. The concentration difference was still equivalent to 0·018 per cent. NaCl ($\Delta = 0·010_5$° C.).

(v) Vapour-pressure measurements have repeatedly confirmed the decrease of osmotic-pressure difference between white and yolk with time. Eggs preserved in isinglass for 5 months showed a mean value 0·022 per cent. NaCl for the vapour-pressure difference. This change, if Grollman's contention were correct, would indicate that the white becomes hypertonic to the yolk, which is unlikely.

(vi) Grollman remarks that Straub's values for the freezing-points of mixtures of white and yolk do not agree with those calculated from the water contents and the original freezing-points. This is scarcely justified, since the discrepancy is usually small, having the mean value 0·007° C. for fresh eggs and only 0·003° C. for preserved eggs. Table IV gives the results of vapour-pressure measurements on mixtures of yolk and white, quantities containing approximately equal weights of water being mixed, excepting in experiment B, when the proportions are given in the table. The vapour pressures of yolk and mixture were determined by direct comparison with pure white. The results, which confirm neither Grollman's criticism nor Straub's experimental figures, are discussed on p. 327.

Table IV. *Vapour pressures of mixtures of white and yolk.*

A.

Column 1: osmotic concentration of yolk, in terms of the osmotically equivalent NaCl solution and the corresponding freezing-point depression.
Columns 2 and 3: osmotic concentrations of white and mixture respectively.
Column 4: theoretical values for column 3, calculated from 1 and 2 and observed water content of substances mixed.
Column 5: discrepancy between observed and calculated values—*i e.* 4 − 3.

	1 Yolk		2 White		3 Mixture observed		4 Mixture calculated		4 − 3	
	% NaCl	Δ	% NaCl	Δ	% NaCl	Δ	% NaCl	Δ	% NaCl	Δ
1	0·879	0·514	0·712	0·416	0·704	0·412	0·773	0·452	0·069	0·040
2	0·910	0·533	0·751	0·439	0 697	0·407	0·806	0·472	0·109	0·064
3*	0·892	0·522	0·702	0·411	0·694	0·406	0·769	0·450	0·070	0·044
4†	0·795	0·465	0·761	0·445	0·706	0·413	0 776	0·454	0·070	0·041
5‡	0·881	0·515	0·708	0·414	0·711	0·416	0 801	0·468	0·090	0·053

* Duck's egg.   † Preserved egg.   ‡ g = 54 (see experiment B).

B.

g is the percentage of total water in the mixture supplied by the yolk.
The total water in the white was 87·1 per cent. and in the yolk 51·0 per cent.

Substance	g	Vapour-pressure observed c_0		Vapour-pressure calculated c_c		$c_0 - c_c$	
		% NaCl	Δ	% NaCl	Δ	% NaCl	Δ
White	0	0·675	0·395	—		—	
Yolk	100	0·897	0·525	—		—	
Mixture 1	59	0 772	0·452	0 805	0·471	0·033	0·019
Mixture 2	42	0·686	0·402	0·768	0 449	0·082	0·048
Mixture 3	33	0·685	0·401	0·749	0 438	0·064	0·037

DISCUSSION.

Most of the foregoing experiments were done at Plymouth in the early summer of 1931. They showed clearly that Grollman's results could not be accepted and that his criticism of the thermal method of determining vapour pressures was groundless, without providing any obvious explanation of his results. They were communicated to Prof. O. Meyerhof, who attempted to repeat Grollman's dialysis experiments, using precisely the same technique. He was able to show that Grollman had neglected to take into account the water in his collodion membranes, and that the results agreed with those obtained by the vapour-pressure method as soon as this factor was allowed for (Meyerhof, 1931). The chief point in Grollman's work is thus explained away, though it is still not clear why, using Meyerhof's thermo-electric apparatus, he consistently found the yolk of preserved eggs to have a higher osmotic pressure than the white.

In the vapour-pressure measurements on mixed yolk and white, the mixture is in all cases osmotically more dilute than calculated; preserved eggs show practically

the same effect. The extent of the disagreement with Straub's data is shown in Fig. 1, which shows roughly the behaviour of mixtures of white and yolk according to vapour-pressure measurements on fresh and preserved eggs. The points determined by Straub were all with mixtures of the whole white and yolk of an egg, of approximate composition S in Fig. 1, and they all lie nearly on the straight lines c and d. The discrepancy is thus about 0·034° C. (Cp. p. 323, footnote.)

The cause of the anomaly is probably the removal of osmotically active substances by adsorption, perhaps as a result of a change in the properties of the egg
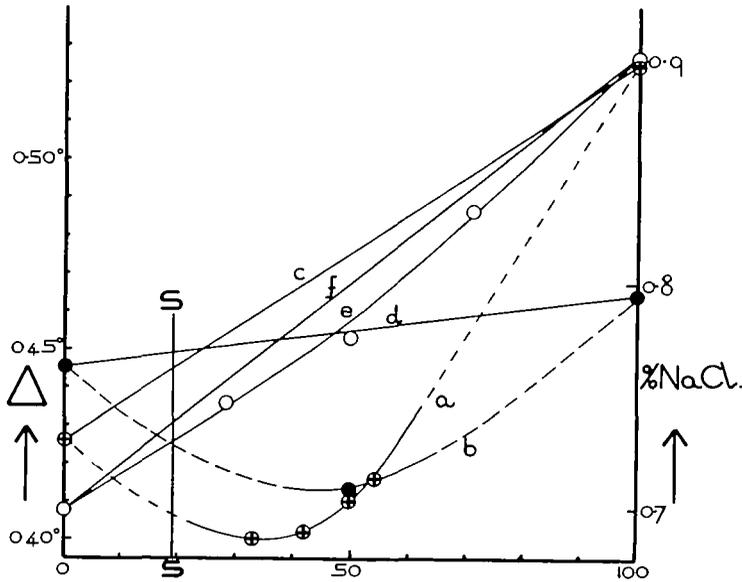

Fig. 1. Vapour pressures of mixtures of white and yolk.

Ordinate: vapour pressures, expressed in per cent. NaCl (right-hand scale) or the corresponding Δ (left-hand scale).
Abscissa: composition of mixture, expressed as per cent. total water contributed by the yolk.
Curves a, b: observed vapour-pressures of mixtures of white and yolk for fresh and preserved eggs respectively. c, d: the theoretical vapour-pressures of these mixtures. e. mixtures of yolk and 0·7 per cent. NaCl.
S represents roughly the composition of the mixtures used by Straub (1929), and the freezing-points determined by him all lie nearly on the lines c and d.

colloids following dilution, change of pH, etc. No serious attempt has been made to disentangle the factors involved, but the experiments in the next section suggest a few possibilities, and in particular they dispose of the possibility that the effect is due to a release of bound water.

The effect has a certain bearing on previous observations, mentioned briefly below. (i) In the fertilised egg there occurs rapid absorption of white by the yolk, during which the yolk approaches the white in osmotic pressure while the white itself becomes rather more dilute (Bialascewicz, 1912; Vladimirov, 1929; see also Needham, 1931, p. 815). There is obviously a decrease in total osmotically active

material, which may be in part due to the effect recorded above. Evidently the absorption of white is not merely a passage of water in the direction of the osmotic gradient (cp. Vladimirov's conductivity measurements, 1929). (ii) The freezing-point changes in the unfertilised egg, and the accompanying water transfer have been studied by Smith and Shepherd (Needham and others, 1931), who found a large discrepancy between the two sets of data, which indicated an increased total osmotic concentration in the system. The present observations only show the importance of the time factor, since the effect of mechanical admixture, although anomalous, in no way resembles the slow changes observed by Smith and Shepherd.

THE STATE OF WATER AND OF CERTAIN ADDED SUBSTANCES IN THE EGG.

Some of these experiments followed naturally from the above discussion, and the remainder form part of a more general programme of work. (i) Egg-yolk was diluted with roughly isotonic sodium chloride solution and the vapour pressures of yolk and diluted yolk were determined by comparison with that of 0·7 per cent. NaCl. The results are given in Table V, and are calculated in the manner of those given in Table IV, B.

Table V. *Dilution of egg-yolk with 0·7 per cent. NaCl solution.*

Substance	g	Observed vapour-pressure c_0		Calculated vapour-pressure c_c		$c_c - c_0$	
		% NaCl	Δ	% NaCl	Δ	% NaCl	Δ
0·7 % NaCl	—	0·700	0·409	—		—	
Yolk	—	0 898	0·526	—		—	
Mixture 1	71	0·832	0·486	0·842	0·493	0 010	0·006
Mixture 2	50	0·774	0·453	0·800	0·468	0 026	0·015
Mixture 3	28	0·746	0·436	0·755	0·442	0·009	0·005

Table VI.

A. Dilution of egg-white with water and 2 per cent. NaCl.
B. Dilution of egg-yolk with water and 2 per cent. NaCl.
The results are calculated in terms of the apparent free water, expressed as a percentage of total water, x.[1]

A.		B.	
Substance added	x	Substance added	x
Water	97·0	Water	102·0
	104·3		105·7
	106·2		99·7
	Mean 102·7		Mean 102·5
2 % NaCl	100 7	2 % NaCl	110·7
	104·6		108·9
	101·7		111·8
	Mean 102 3		Mean 110·5

[1] Cp. Hill (1930 c). If g gm. yolk, containing fg gm. water (determined by drying the yolk at 105°C.), is mixed with g_s gm. of a sodium chloride solution containing $f_s g_s$ gm. water, and if x is the

Table VI gives the results of similar experiments in which the effect of adding diluting white and yolk with water and with 2·0 per cent. NaCl was determined.

(ii) For comparison with the above results, the apparent free water was determined independently by addition of solid sodium chloride, and in one case of solid urea. The amount of substance added was sufficient to produce a change in osmotic concentration roughly equivalent to 0·5 per cent. NaCl (Table VII).

Table VII. *Apparent free water on adding solid NaCl or urea.*[1]

Substance added	x	
	White	Yolk
NaCl	100·1	101·1
NaCl	101·7	—
NaCl	96·2	99·8
NaCl	102·2	108·6
	Mean 100·3	Mean 103·2
Urea	96·3	125·6

The above values for yolk are selected, since high values were rather often obtained, probably because of the difficulty in ensuring complete mixing of the added substance. 99·8 per cent. is the *minimum* result.

Table VIII. *Dilution of yolk and white with urea, glucose and sodium lactate solutions.*

$g \sim 50$ in all cases.
— sign in the last column indicates removal of substance from solution.
The sodium lactate solution was prepared from B.D.H. 50 per cent. solution and had a vapour pressure equivalent to 1·5 per cent. NaCl, or a Δ of 0·878° C.

Number of experiments	Solution added	Substance	$(c_0 - c_0)$	
			% NaCl	Δ
1	2·0 % urea	Yolk	− 0·029	− 0·016
4	1·8 % urea	Yolk	− 0·016	− 0·0094
2	1·5 % urea	White	− 0·001	− 0·0006
1	6·0 % glucose	Yolk	+ 0·002	+ 0·0011
1	4·2 % glucose	White	+ 0·009	+ 0·005
2	2·0 % Na lactate	Yolk	− 0·036	− 0·021

apparent free water in the yolk (as fraction of the total water), then it is easy to show that

$$x = \frac{f_s g_s (c_s - c_m)}{fg (c_m - c)} = \frac{f_s g_s}{fg} \frac{\theta}{\phi},$$

where c_s, c_m and c are the osmotic concentrations of the sodium chloride solution, the mixture and the pure yolk respectively, and θ, ϕ are respectively the galvanometer deflections produced when the following vapour-pressure measurements are made:

θ: Yolk against Mixture.
ϕ: Mixture against NaCl solution.

Since the true free-water fraction cannot be greater than 1, the above result must be incorrect if $x > 1$—e.g. because of removal of osmotically active substances. If $x < 1$, a fraction $(1 - x)$ of the water may be "bound" or osmotically inactive, or else osmotically active substances may be liberated from the yolk colloids on dilution.

[1] The effect of adding a known weight of the solid substance to egg-white or yolk is compared with the effect of adding an equal amount of the same substance to an isotonic pure NaCl solution or Ringer. Then x can be calculated in terms of the observed galvanometer deflections—cp. p. 328, footnote.

(iii) Approximately isotonic solutions of other substances were added with the object of detecting by vapour-pressure measurements any removal of these substances from the continuous phase (Table VIII).

DISCUSSION.

Table V. An appreciable amount of electrolyte is removed from solution on dilution, but the amount is much less than when yolk is diluted with white. This is shown in Fig. 1.

Table VI. Dilution with water causes a slight increase in total amount of osmotically active substances in the system. Dilution is not therefore in itself responsible for the effect of adding white or a salt solution to egg-yolk. The values for the apparent free water, moreover, give no precise indication of the amount of bound water present, although this is probably very small. Dilution with 2 per cent. NaCl produces nearly the theoretical decrease in vapour pressure of white, and a considerably smaller decrease in that of the yolk.

Table VII. Addition of solid NaCl produces effects of a similar kind, again indicating some adsorption by the yolk, and again leaving the question of bound water undecided. But the results, as far as they go, are contrary to Rubner's (1922) value for the bound water of egg-white and in agreement with those of Hill (1930 c). They disagree with Hill's single experiment on yolk, which gave 85 per cent. for the apparent free water.

The experiments on urea given in Tables VII and VIII will be discussed in a later paper in connection with the state of urea in blood. It may be mentioned here that the effect on egg-white is that observed by Hill (1930 c), and that its apparent removal from solution when added to yolk reminds one of its effect on blood (Hill, 1930 c). Two preliminary experiments showed that, when dissolved in a 2 per cent. lecithin emulsion, urea produced a normal effect on the vapour pressure.

Sodium lactate is also largely removed; calculated as free water, the results would be 136 and 134 per cent. Glucose, which one would expect to be removed by the lipoids present in the yolk (see Scott, 1916), apparently remains entirely in solution.

SUMMARY AND CONCLUSIONS.

1. The existence of a real osmotic difference between white and yolk of the hen's egg has been confirmed.

2. Hill's vapour-pressure thermopiles are shown to be reliable when used with viscous protein solutions such as egg-yolk.

3. The vapour-pressure changes which occur on mixing white and yolk indicate a removal of osmotically active substances. They do not agree with the freezing-point determinations of Straub.

4. The bearing of this result on the osmotic changes occurring in the fertilised and unfertilised egg is discussed.

5. The effects of diluting white and yolk with water and with various salt solutions is studied from the point of view of 3 (above) and in relation to the problem of bound water. It is concluded that the amount of bound water in both white and yolk is small. The effect of solid sodium chloride on the vapour pressure of these substances confirms this conclusion.

6. Urea appears to dissolve in egg-white with normal depression of vapour pressure; urea and sodium lactate are largely removed from solution when added to yolk. Glucose is not so removed.

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